

probe array can be highly uniformly controlled. This can improve the uniformity of the hybridization across the microarray.

[0156] An embodiment of a hybridization apparatus includes a hybridization assembly and a target liquid motion inducer in a hybridization chamber. The hybridization assembly comprises a reaction chamber (or hybridization chamber) to confine and allow interaction or binding of a target liquid to an array of probes deposited on an inner surface of the reaction chamber. The hybridization assembly may comprise a substrate slide, a gasket layer and/or a middle slide, and a cover slip. The cover slip, the gasket layer and/or the middle slide, and the substrate slide can be fastened together to form a watertight hybridization chamber. FIG. 14 shows an example of a "sandwich" hybridization chamber. For hybridization, the chamber is filled with the target liquid. The substrate slide has an array of probes deposited on the substrate slide surface facing the hybridization chamber. A spring steel slide holder or other clamping mechanism may be used to maintain pressure in the slide stack, as shown in FIG. 15.

[0157] The middle slide has a through opening which can be precisely formed to be slightly larger than the outer dimensions of an array of probes on the substrate slide so that the array of probes is positioned inside the opening when the middle slide is placed on the substrate slide. Alternatively, the middle slide may have a plurality of openings that match a plurality of arrays of probes on the substrate slide. The thickness of the middle slide may be, for example, from 10  $\mu\text{m}$  to 5 mm. The substrate slide and the middle slide can be made of any suitable materials including glass, silicon, polymer, plastic, ceramic, metal, wood, rubber, silicone rubber, etc.

[0158] When the middle slide is made of a relatively hard material such as glass, ceramic or metal, a gasket layer may be attached to the surface of the middle slide that contacts the substrate to serve as a seal (FIG. 16). This gasket layer should ideally be made of softer and hydrophobic material such as silicone rubber, polytetrafluoroethylene, Teflon®, or polydimethylsiloxane (PDMS). The method of attachment can be lamination, injection molding gluing or any other means, or the gasket layer can be held in place by the clamping force. If both the substrate and the middle slide are very flat, it is also possible to make the gap between the middle and substrate slide surfaces water tight by simply making both surfaces highly hydrophobic and pressing the two tightly together.

[0159] During hybridization, the middle slide is placed on the substrate slide with the array of probes positioned inside the opening. The middle and substrate slides are tightly pressed against each other to provide a watertight seal preventing fluid leakage through a gap between the two slides. Atmospheric pressure is often sufficient to maintain the seal. For added assurance, a spring slide holder designed to clamp the slides together by applying pressure to the outer surfaces can be used to maintain the pressure, as illustrated in FIG. 15. In this way, the opening through the middle slide and the substrate forms wells on the microarray into which one or more sample or target liquids are introduced using a precision liquid delivery device such as a pipette.

[0160] The volume of the sample liquid may be controlled so that the liquid surface in the "wells" created by the middle

slide and the gasket layer (as illustrated in FIG. 14) is below the upper surface of the middle slide. Because both the volume of the sample liquid and the dimension of the middle slide opening can be precisely controlled, the height of the liquid inside the well, and thus the effective target hybridization volume can be precisely metered. In this way, the chip-to-chip hybridization variation can be minimized. A cover slip can be placed on top of the middle slide to reduce evaporation.

[0161] For another embodiment of this device, the cover slip and the middle slide can be an integrated piece, as shown in FIG. 17. The integrated cover slip has a well that is slightly larger than the outer dimensions of an array of probes on the substrate slide. When the cover slip is aligned with the microarray substrate slide, the well covers the array of probes on the substrate slide. The cover slip may have a plurality of wells that match a plurality of arrays of probes on the substrate slide. The cover slip can be made of, for example, plastic, polymer, glass, silicon, metal, ceramic, wood, rubber, silicone rubber, or any other suitable materials. The wells can be formed by machining, etching, molding or other suitable processes. A very thin gasket layer can be bonded to the lower surface of the integrated cover slip, which provides a seal at the interface between the integrated cover slip and the substrate slide.

[0162] In another embodiment shown in FIG. 18, the cover slip can be flat and have a thick gasket layer bonded to the bottom surface. The gasket layer has openings which form the wells.

[0163] In various embodiments, during hybridization, the cover slip is placed upside down and such that the wells face up, as shown in FIG. 19. Sample or target liquid is added to the wells (FIG. 19a). Then the microarray substrate slide is placed upside down on the cover slip, i.e. the surface having the microarray probes deposited thereon faces the cover slip. The cover slip and the substrate slide can be pressed tightly against each other to squeeze out air bubbles from the interface between the slide and the gasket (FIG. 19b). Before hybridization, the entire assembly is inverted to position the microarray substrate underneath the cover, thereby allowing the target fluid to contact the array of probes, as shown in FIG. 19c. A spring clamp or steel slide holder similar to the one illustrated in FIG. 15 can be used here to maintain pressure between the cover slip and the substrate slide.

[0164] In another embodiment shown in FIG. 20, the cover slip is a layer of liquid deposited over the sample solution, thereby forming a "lid" or layer to prevent evaporation of the sample liquid. This liquid layer can be selected to be immiscible and non-reactive with the sample solution. The liquid cover layer can also be deposited while in liquid form, and hardened into solid or semi-solid form after deposition to form the "lid."

[0165] In some embodiments, the cover slip and the middle slide can be single use consumables or they can be reused for many different hybridizations after washing.

[0166] The movement of the target liquid can be created by forces such as, for example gravity, centrifuge force, magnetic force, sonic force, electronic force, Lorentz force, thermodynamic force, pneumatic force, or/and mechanical force, as described in greater detail below.